

ETHOSOMES: A NOVEL APPROACH TO ENHANCEMENT THE BIOAVAILABILITY OF POORLY SOLUBLE DRUGS

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ABSTRACT

Ethosomes are novel carrier systems used for delivery of drugs that have low penetration through the biological membrane mainly skin. Ethosomes are soft and malleable lipid vesicles containing phospholipid, high proportion of alcohol and water, to enhance the delivery of active pharmaceutical agents. Ethosomes have higher penetration rate through the skin as compared to the liposomes. The increase permeation of ethosomes is due to its higher ethanolic content, are able to encapsulate and deliver the highly water soluble drug through the skin.

Ethosomes provide a number of important benefits including improving the drug efficacy, enhancing bioavailability, patient compliance and reducing the total cost of treatment. Enhance delivery of bioactive components through the skin and cellular membrane by an ethosomal novel carrier opens numerous challenges and opportunities for the research and future development of novel improved therapies.

Keywords: Ethosomes, Liposomes, Bioavailability.

INTRODUCTION

Human skin is an effective, selective barrier for the permeation of water soluble drug, although the skin as a route for delivery, having many advantages like avoidance of first-pass metabolism, reduce fluctuations in plasma drug levels, drug targeting for a local effect and good patient compliance.¹ Water soluble molecules and drugs are commonly not able to cross the skin because the skin is a natural barrier to water. The stratum corneum is composed of insoluble bundled keratins surrounded by a cell envelope, stabilized by cross-linked proteins and covalently bound lipids. Several advance technology have been made to overcome skin barrier properties. Examples physical means such as iontophoresis, sonophoresis, microneedles (are relatively complicated to use and will affect patient compliance), chemical means, using permeation enhancers and biochemical means such as, liposomal vesicles and enzyme inhibition.² The use of chemical enhancers such as surfactants and organic solvents (induce irritation, cause damage, and reduce skin barrier function).³ One such approach is the use of vesicular systems. In the past decade, topical delivery of drugs by liposomal formulation has evoked considerable interest. Deformable liposomes and transferosomes were the first generation of elastic vesicles introduced and were reported to penetrate intact skin while carrying a therapeutic concentration of drugs.⁴ Ethosomes were developed by Touitou, as additional novel lipid carriers composed of ethanol, phospholipids, and water. They are reported to improve the skin delivery of various drugs.⁵

Ethosomes are non-invasive delivery carriers that enable drugs to reach the deep skin layers and / or the systemic circulation. Ethosomal carriers are systems containing soft vesicles. It is composed by the phospholipid (Phosphotidyl choline), high concentration of ethanol and water. The high concentration of ethanol makes the ethosomes unique, as ethanol also enhance the permeation of drug in to the skin (stratum corneum). The size range of ethosomes may vary from tens of nanometers to microns.⁶ The synergistic effects of combination of phospholipids and high concentration of ethanol in vesicular formulations have been suggested to be responsible for deeper distribution and penetration in the skin lipid bilayers.⁷

Ethosomes are mainly used for the delivery of drugs through transdermal route. Also, because of their high ethanol concentration, the lipid membrane is packed less tightly than the conventional vesicles, although it has equivalent stability, allowing a more malleable structure and improves the drug distribution ability in the stratum corneum lipids. Lipophilic drugs can pass through the skin but the drugs which are hydrophilic in nature can't pass through. Water soluble drugs either show very less or no permeation.

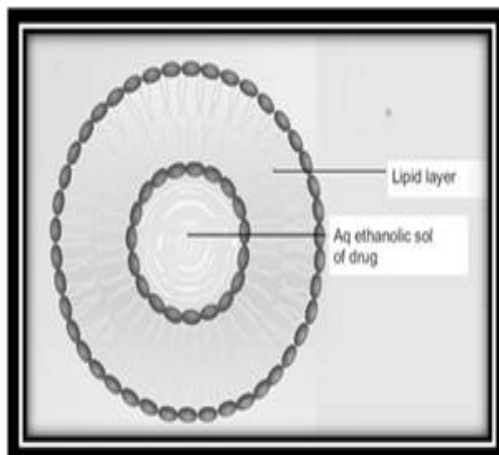


Fig. Structures of Ethosomes

ADVANTAGES OF ETHOSOMAL DRUG DELIVERY⁸

- Enhanced drug permeation through skin for transdermal drug delivery
- Make possible the delivery of large molecules (peptides, protein molecules)
- Raw material used in the formulation of ethosomes, are non-toxic in nature
- They produce high patient compliance
- The ethosomal system is passive
- They are non-invasive method
- They are commercially available
- Ethosomal drug delivery system are widely applied in Pharmaceutical, Veterinary, Cosmetic fields
- Method of the preparation is simple as compare to Iontophoresis, Phonophoresis and other complicated methods.

MECHANISM OF PENETRATION

- Ethanol interacts with the lipid molecules in the polar head group region resulting in a reduction in the transition temperature of the lipids in the stratum corneum, increase their fluidity and decrease the density of the lipid multilayer. This is followed by the "ethosome effect", which includes lipid penetration and permeation by the opening of new pathways, due to the malleability and fusion of ethosomes with skin lipids, resulting in the release of the drug into the deep layers of the skin.
- Ethanol may also provide vesicles with soft flexible characteristics, which allow them to penetrate more easily into the deeper layers of the skin. The release of the drug in the deep layers of the skin and its transdermal absorption could then be the result of a fusion of ethosomes, with skin lipids and drug release at various points along the penetration pathway^{9,10}

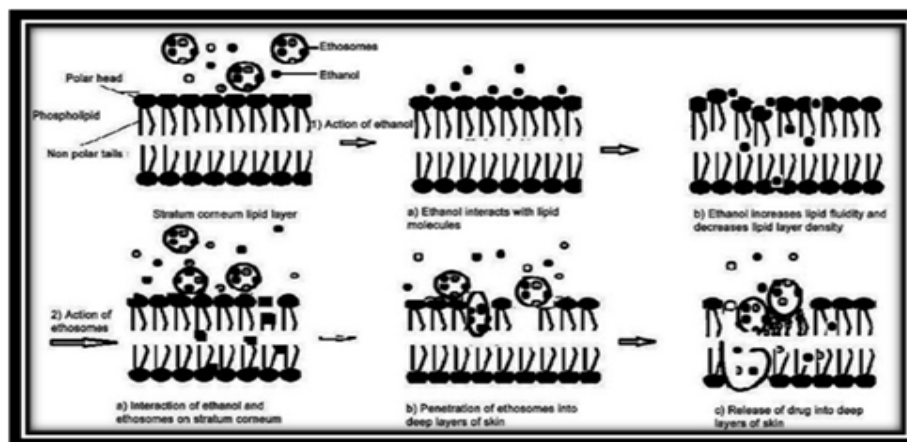


Fig. Mechanism of drug Penetration

Ethosomes increase drug absorption through skin in two phases

- Ethanol effect
- Ethosomes effect

➤ Ethanol effect

Ethanol acts as a penetration enhancer which increases the movement of drug through the skin. Penetration of ethanol into intercellular lipids, lead to increases the fluidity of cell membrane lipids and decrease the density of lipid multilayer of cell membrane¹¹

➤ Ethosomes effect

Increased cell membrane lipid fluidity caused by ethosomes containing ethanol and it will result into the improved skin permeability. So the ethosomes very easily permeates inside the deep skin layers, where ethosomes

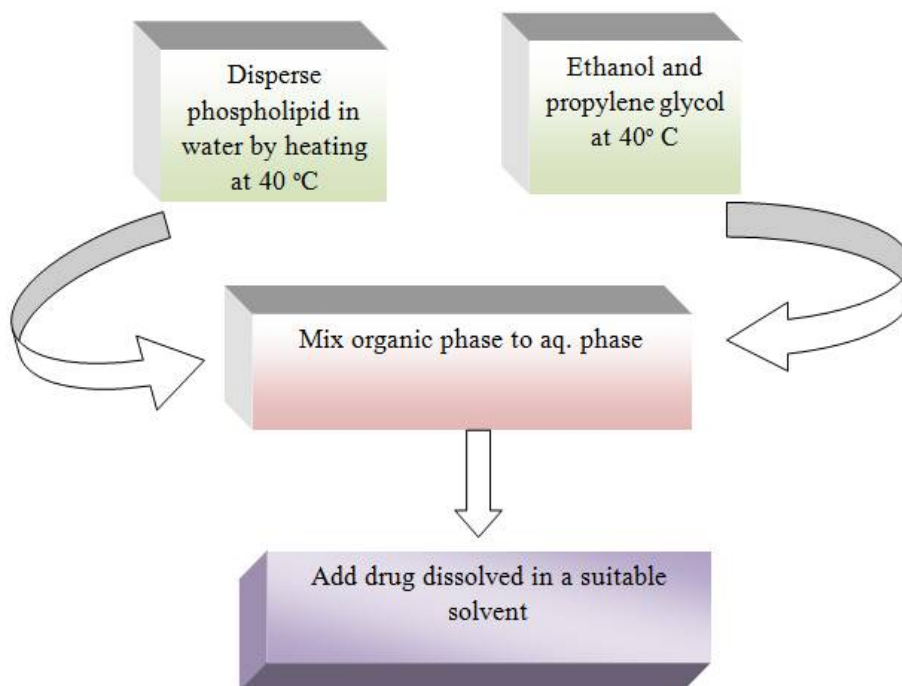
DIFFERENT ADDITIVES EMPLOYED IN FORMULATION OF ETHESOMES

Class	Example	Uses	References
Phospholipid	<ul style="list-style-type: none"> • Soya phosphatidyl choline • Egg phosphatidyl choline • Dipalmityl phosphatidyl choline • Distearyl phosphatidyl choline 	Vesicles forming component	11
Polyglycol	Propylene glycol	Act as a skin penetration enhancer	12
Alcohol	<ul style="list-style-type: none"> • Ethanol • Isopropyl alcohol 	For providing the softness for vesicle membrane As a penetration enhancer	13
Cholesterol	<ul style="list-style-type: none"> • Cholesterol 	For providing the stability to vesicle membrane	14
Dye	<ul style="list-style-type: none"> • Rhodamine-123 • Rhodamine red • Fluorescence Isothiocyanate (FITC) • 6-Carboxy fluorescence 	For characterization study	15
Vehicle	Carbopol D934	As a gel former	12

METHODS OF PREPARATION OF ETHESOMES- Ethosomes are prepared by two method

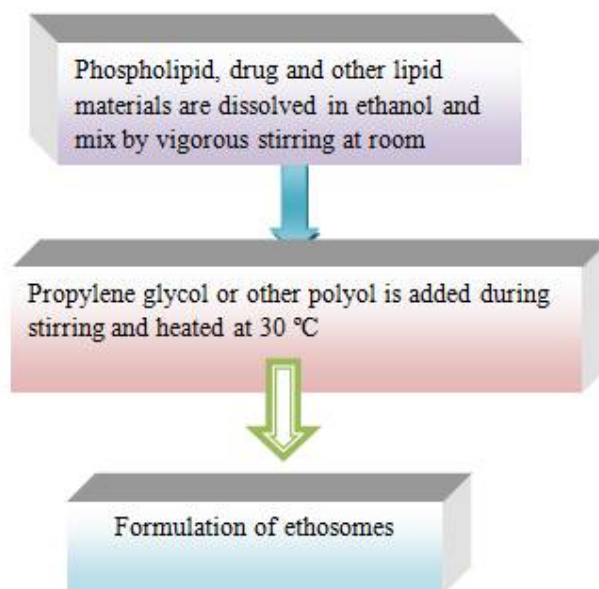
➤ Hot method

The drug is dissolved in ethanol and propylene glycol mixture which is heated at 40 °C and the phospholipid is dispersed in water at 40°C, the organic phase is added to the aqueous with stirring. After mixing the preparation is sonicated at 4°C for few minutes, the formulation is then homogenized at 15,000 psi pressure to get nano-sized ethosomes¹⁶



➤ **Cold method**

The drug, phospholipids and other lipid materials are dissolved in ethanol in a covered vessel and are mixed by vigorous stirring at room temperature. Propylene glycol or other polyol is added during stirring and mixture is heated at 30°C in a water bath. The water is heated at 30 °C in a separate vessel and added to the above mixture with stirring for five minutes in a covered vessel. The vesicle size of ethosomal formulation can be decreased to desired extent using sonication or extrusion method. Finally, the formulation should be stored under refrigeration.¹⁷



VARIOUS METHODS OF CHARACTERIZATION OF ETHOSOMES

➤ **Size analysis of vesicular systems**

The mean size of ethosomal colloidal suspension was analyzed by dynamic light scattering technique with a Zetasizer 3000 HSA (Malvern Instruments, Malvern, UK). The sample was placed in quartz cuvette and size measurements were carried out at a scattering angle of 90°. All observations were recorded in triplicate for each formulation.

➤ **Vesicle shape**

Shape and morphology of the ethosome vesicles were investigated using transmission electron microscopy. Formulation diluted with water was adsorbed onto a grid with carbon-coated formvar film that was attached to a metal specimen grid. Excess sample was blotted off and the grid was covered with a small drop of staining solution (2% w/v uranyl acetate). It was left on the grid for few minutes and excess solution was drained off. The grid was allowed to dry thoroughly in air and sample was examined in the transmission electron microscope¹⁸.

➤ **Measurement of entrapment efficiency¹⁹**

For determination of entrapment efficiency the vesicles were separated from the untrapped or free drug by Sephadex G-25 minicolumn centrifugation method. For preparation of minicolumn, Sephadex G-25 (2 g) was suspended in 100 ml saline and was kept overnight. Subsequently supernatant was decanted and the swollen gel was poured in 1 ml syringe and centrifuged to get packed column free from saline. Formulation was placed on the top of Sephadex column, and the formulation free from untrapped drug was collected from the bottom. Separated formulation was lysed using 10% v/v Triton X-100 and drug content was determined using HPLC method. The Sephadex column was covered to minimize the evaporation of ethanol from the hydroalcoholic ethosomal formulation. However, there is no influence of this small change in the composition of formulation on drug entrapment efficiency. Actually the total amount of drug entrapped in the vesicles would not change as the formulation elutes through the column.

The percent entrapment was calculated using the formula

% Entrapment= amount of drug in sediment/amount of drug added ×100.

➤ **In vitro permeation of drug through skin²⁰**

In vitro permeation was determined using flow through diffusion cell system consisting of 16 channel peristaltic cassette pump (Ismatech, Switzerland), a circulating water bath (Hakke, Germany), a fraction collector (ISCO Retriever IV, US) and flow through diffusion cells, similar to the setup we reported earlier. Adult Chinese female skin was used for the experiment. For the preparation of the epidermis for experiment, skin along with epidermis was immersed in water at 60 °C for 2 min and epidermis was carefully peeled off and stored at -80 °C until use. Prior to experiments, skin was thawed and hydrated with saline solution containing 1% v/v antibiotic antimycotic solution. Epidermis was mounted between the donor and receptor compartments of flow through cells and excess part of the skin was trimmed off. Phosphate buffer solution containing 1% v/v antimycotic solution was filled in the reservoir bottle. Receptor solution was thoroughly degassed to prevent the formation of bubbles beneath the epidermis. Formulation (1 ml) was placed in the donor compartment and covered with parafilm to prevent contamination and evaporation. Ambient temperature of the cells was maintained at 37 °C by circulating water bath. The receptor solution was pumped by peristaltic cassette pump continuously through the receptor compartment and drained into sample collection test tubes located in the fraction collector. Samples were collected at various time intervals and analyzed for amount of ketoprofen using HPLC. The graph was plotted between cumulative amount of drug permeated versus time and with the slope of graph transdermal flux (J) was calculated. Steady state drug plasma concentration (P_{ss}) in vivo through the skin can be predicted using the following equation, if the drug were in a patch with area (A) of 50 cm².

➤ **Vesicle size and Zeta potential**

Particle size and Zeta potential of the ethosomes can be determined by dynamic light scattering (DLS) and photon correlation spectroscopy (PCS). Size and size distribution were determined by dynamic light scattering (DLS) using a computerized inspection system. Surface morphology was determined by TEM, a drop of the sample was placed on a carbon-coated copper grid and after 15 min it was negatively stained with 1% aq. solution of phosphotungstic acid. The grid was allowed to air dry thoroughly and samples were viewed on a transmission electron.²¹

➤ **In vitro drug permeation study**

The in vitro permeation study was carried out by using Franz diffusion cell with egg membrane. The study was performed with phosphate buffer saline (pH 7.4). The formulation was placed on the upper side of skin in donor compartment. The temperature was maintained at $37 \pm 2^\circ$. Samples were withdrawn after every hour from the receptor media through the sampling tube and at the same time, fresh media was added to receptor to make sink condition. Withdrawn samples were analyzed for drug constant using UV/Vis spectrophotometer^{22,23}.

➤ **Stability studies**

Formulations were stored at $4 \pm 2^\circ$, 8° and at room temperature. Percent drug entrapment was determined at different time intervals. The stability of vesicles can be determined by assessing the size and structure of the vesicles over time. Mean size is measured by DLS and structure changes are observed by TEM¹².

APPLICATION OF ETHOSOMES AS A DRUG CARRIER

➤ **Delivery of Anti-Viral Drugs**

Antiviral agent acting on acquired immunodeficiency virus. ethosomes could increase the transdermal flux, prolong the release eg. Zidovudine^{24,25}

➤ **Transdermal Delivery of Hormones**

Oral administration of hormones is associated with problems like high first pass metabolism, low oral bioavailability and several dose dependent side effects. Ethosomes reduce these problem and improved drug permeation through skin. Eg testosterone (Testoderm patch, Alza).²⁶

➤ **Delivery of anti-parkinsonism agent**

Skin permeation potential of ethosomal-THP formulation and its use for better management of Parkinson disease.

➤ **Delivery of Antibiotics**

Topical delivery of antibiotics is a better choice for increasing the therapeutic efficacy of these agents. Conventional oral therapy causes several allergic reactions along with several side effects. Ethosomes can circumvent this problem by delivering sufficient quantity of antibiotic into deeper layers of skin.¹²

➤ **Drug targeting**

Selective delivery of drug to desired side for prolong period of time. Eg NSAIDS(Diclofenac).

➤ **Improved Bioavailability**

Increase skin permeation Improved in biological activity two to three times and Improved in Pharmacodynamic profile. Eg Acyclovir.

➤ **Improved patient compliance**

Provide controlled release, Improved transdermal flux, Improved patient acceptability and Biologically active at dose several times lower than the currently used formulation. Eg Trihexyphenidyl hydrochloride, Lamivudine, Azelaic acid.

➤ **Improved biological activity**

Cannabidol, Insulin, Zidovudine, Azelaic acid.²⁷

CONCLUSION

It can be easily concluded that ethosomes can provide better skin permeation than liposomes. Ethosomes are characterized by simplicity in their preparation, safety, efficacy and tailored for enhanced skin permeation of active drugs. The main limiting factor of transdermal drug delivery system i.e. epidermal barrier can be overcome by ethosomes to significant extent.

Ethosomes have been tested to encapsulate hydrophilic drugs, cationic drugs, proteins and peptides. Ethosomal carrier opens new challenges and opportunities for the development of novel improved therapies.

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